REMARKS

I. Amendments to the Claims

Applicants have amended the claims to more particularly define the invention taking into consideration the outstanding Office Action. In particular, Applicants have amended claims 26, 45, 47, 48, 50, 66 and 68 and have canceled claims 31, 33, 35 and 61 from the present application. New claims 69 and 70, reciting particular embodiments of the invention, have been added.

The amendments to the claims, including cancellation of claims, have been made without prejudice or disclaimer to any subject matter recited or canceled herein. Applicants reserve the right to file one or more continuation and/or divisional applications directed to any canceled subject matter. No new matter has been added, and entry of the foregoing amendments to the claims is respectfully requested.

II. Response to Claim Objections

Claims 31, 66 and 68 have been objected to for containing informalities.

In response, the claims have been canceled (claim 31) or amended as required by the Examiner (claims 66 and 68). Accordingly, Applicants respectfully request reconsideration and withdrawal of the objections.

III. Response to Claim Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 47-49 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for reciting an undefined ratio.

Claims 47 and 48 have been amended to clarify the second ratio. Applicants submit that the claims as amended particularly point out and distinctly claim the subject matter Applicants regard as the invention. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

IV. Response to Claim Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 26-68 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to meet the enablement requirement. This rejection has been carefully considered but

is most respectfully traversed in view of the amendments to the claims and the following remarks.

Initially, Applicants note that the reference referred to hereinafter as Giesling et al., 2009, is the recent publication of Prof. Giesing cited by the Examiner in the November 12, 2009, Office Action.

In said publication, the abbreviation AOX is short for antioxidant enzymes, i.e. MNSOD, TXNRD1 and GPX1 as recited in the claims. CCC is short for circulating cancer cell clusters and CEC is short for circulating epithelial cells. Both CCC and CEC qualify as disseminated cancer cells.

Applicants' comments with respect to various aspects of the enablement rejection are as follows:

1. *Any subject versus human subject*

On page 8 of the Office Action, the Examiner asserts that the specification does not reasonably provide enablement for the use of the method for a subject other than a human subject. As amended herein, all independent claims are directed to a method for investigating a body fluid for disseminated cancer cells in a human subject.

2. *Bone marrow versus blood*

The claims are drawn to a method for investigating body fluids for disseminated cancer cells.

In contrast to solid tumors, disseminated cancer cells circulate in the body of an individual. This usually takes place via endogenous transport organs, especially body fluids, in particular blood (see, for example, page 5, line 36 to page 6, line 4 of the specification) and also bone marrow, the compartment which is nearest to blood.
Applicants have submitted a review article published by the group of K. Pantel in order to illustrate the history of tumor cell findings in bone marrow (submitted with the response filed on April 13, 2009).

It is important to note that the present invention is based on the finding that the migration of cancer cells away from the primary tumor and entry into the systemic vasculature and lymphatics is connected with the development of specific gene expression traits. More specifically, the invention is based on the finding that such disseminated cancer cells over-express genes involved in anti-oxidative protection. While not wishing to be bound

by theory, the inventors believe that said antioxidant overexpression in disseminated cancer cells can be described as a survival and defence mechanism required in an atypical environment. This is stated in the inventors' recent publication cited by the Examiner: "Finally, AOX overexpression in CCC can be described as a survival and defence mechanism required in an atypical environment" (see page 9, paragraph bridging middle and right hand columns, in Giesing et al., 2009).

Although the specification does not provide a working example for the investigation of bone marrow there is no reason to believe that the skilled person would not have been able to practice the invention on bone marrow. This is confirmed in Prof. Giesing's Declaration of record under item 5.4.

Applicants note that claim 28 is specifically directed to the embodiment wherein the body fluid is blood.

3. *Protein versus mRNA and RT-PCR versus microarray*

The Examiner asserts that the specification does not reasonably provide enablement for measuring protein levels to indicate the presence of disseminated cancer cells in the body fluid.

Protein expression is usually directly related to mRNA expression. Keeping in mind that the continuing elevated (protein) expression of AOX acts as a survival and defence mechanism in CCC required in an atypical environment prone to escape from immune surveillance there is no reason to assert that the skilled person would not be able to practice the invention by measuring the expression of the genes at stake on a protein level. Suitable methods for measuring protein levels are well known to those skilled in the art and some of these methods are described in the specification (see, for instance, page 12, line 35 to page 14, line 16).

Applicants note that claim 42 is directed to the embodiment wherein determining the expression of the at least 2 genes comprises determining mRNA expressed by the gene. Similarly, claims 49, 54 and 66 recite said limitation.

On pages 26 and 27 of the office action the Examiner alleges that using a microarray to perform the method of the invention did not generate the necessary sensitivity and specificity to detect circulating cancer cells. The Examiner refers to Giesing et al., 2009, page 3, right column, last full paragraph.

However, there seems to be a misunderstanding. The publication does not disqualify microarray technology as a suitable means for determining the expression of the genes at stake. It is only with respect to the testing of 67 candidate RNAs that the results obtained with medium density microarrays and quantitative real-time RT-PCR were found to show a poor correlation. Thus, the publication compares microarray testing with RT-PCR, giving preference to the latter method.

Moreover, the application teaches a number of techniques suitable for determining gene expression. These are well-known in the art and may be used instead of RT-PCR (compare page 20, line 25 to page 25, line 13).

4. Any MNSOD/TXNRD1/GPX1 gene versus the use of primers having SEQ ID NO: 1, 2, 4, 5, 7, or 8

The Examiner asserts that the specification does not reasonably provide enablement for determining the expression of any manganese superoxide dismutase gene, any thioredoxin reductase gene, or any glutathione peroxidase gene. More specifically, the Examiner asserts that applicant has explicitly defined the term "manganese superoxide dismutase" to encompass both MNSOD and CuZnSOD although one skilled in the art would never consider the term "manganese superoxide dismutase" to encompass CuZnSOD. Applicants disagree.

Given the well-accepted general meaning of the term "manganese superoxide dismutase" those skilled in the art would understand the paragraph bridging pages 14 and 15 of the specification to tell the reader two things about manganese superoxide dismutases. First, these enzymes catalyze the decomposition of superoxide free radicals to form hydrogen peroxide. Second, these enzymes belong to enzyme class 1.15.1.1. However, those skilled in the art would not read this paragraph as defining the term "manganese superoxide dismutase" to include all enzymes of class 1.15.1.1. This is further corroborated by the fact that the specification clearly teaches that there are at least three different superoxide dismutases in human tissues including the cytoplasmic Cu/Zn superoxide dismutases and the mitochondrial manganese superoxide dismutase (MNSOD for short). See page 1, line 36, to page 2, line 4 of the application. Thus, the specification, too, clearly distinguishes between MNSOD and other superoxide dismutases.

The Examiner also asserts that the specification fails to be enabling for determining any MNSOD gene, any TXNRD1 gene, and any GPX1 gene from any species as there are different isoforms of MNSOD, TXNRD1 and GPX1.

First Applicants note that all claims recite the human version of said genes.

Applicants further note that the Examiner states that the primers of SEQ ID NO: 7 and 8 are capable of amplifying genes from a number of different species (see page 18 of the office action). Assuming that in a human subject various isoforms of the genes at stake are expressed and further assuming that the Examiner is correct in that different isoforms are amplified when said primers are used the working examples described in the present specification, the results reported in the declaration of Prof. Giesing and the results described in Giesing et al. 2009 clearly show that the method of the present invention is enabled for any one of said isoforms supposed to occur in humans.

5. *Any method of cancer cell isolation versus using a screen with a mesh or pore width of about 20 μm*

The Examiner seems to assert that the specification does not reasonably provide enablement for any method of obtaining a cell-containing fraction from the body fluid with enrichment of cancer cells.

The specification teaches that methods for isolating cancer cells are well-known in the art. Immunospecific adsorption methods, microdissection methods, density gradient methods or filtration methods are given as examples (page 7, lines 15 to 21). A skilled person would have known how to isolate cancer cells without undue experimentation.

6. *Any test sample A' versus test sample A' from patients' own MNCs*

The Examiner asserts that the specification does not reasonably provide enablement for the use of any further cell-containing fraction and comparing the expression in the cell-containing fraction with the expression in the further cell-containing fraction.

Applicants note that the claims 26 and 48 require that the further cell-containing fraction is derived from the same body fluid as the cell-containing fraction. However, while the cell-containing fraction is obtained with enrichment of cancer cells, the further cell-containing fraction does not involve the enrichment of cancer cells. Thus, if cancer cells are present in the body fluid under investigation the claims at stake are commensurate in scope with the test principle of the invention, i.e. determining whether enrichment of cancer cells is

associated with a measurable increase in MNSOD, TXNRD1 and GPX1 expression (see page 29, lines 33 to 36 of the application).

Applicants note that claim 49 recites that the body fluid is blood and the cell-containing fraction and the further cell-containing fraction comprise mononuclear cells. Claim 45 has been amended accordingly.

7. *Any elevated gene expression versus gene expression higher than a defined limit*

The Examiner asserts that the specification does not reasonably provide enablement for the use of an undefined average gene expression from subjects not having cancer. More specifically, the Examiner appears to believe that the claimed method is enabled only if a limit for the gene expression (which is the average plus one stranded deviation) is determined on healthy subjects.

However, determining such a limit is a matter of validation, but not a matter of practical application of the method. Once a particular test system has been set up it is expedient to run said system on healthy individuals in order to determine the average expression of the genes at stake in cells from the body fluid of said healthy individuals. Using such a limit is an advantage (as it helps avoiding false-positive results), but not a requirement for the method to be enabled.

A skilled person would have readily recognized that once said average expression in healthy subjects has been determined for a given test system the claimed method can be carried out without the need for repeating said determination. It would make no sense if each time the method is used for investigating a body fluid from a patient having or suspected of having cancer it must also be carried out on a healthy subject.

The current amendment reflects that a comparison with a pre-determined average gene expression (ratio) in subjects not having cancer is expedient and sufficient to enable the skilled person to determine the presence of disseminated cancer cells in the body fluid (i.e. in case of a significantly higher ratio of expression in the cell-containing fraction to the expression in the further cell-containing fraction as compared to the average expression in subjects not having cancer (claims 47 and 48); or in case of a significantly higher expression in the cell-containing fraction as compared to the average expression in subjects not having cancer (claims 50 and 66)).

8. *Tumor diagnosis and risk estimation for metastasis versus detection of*

disseminated cancer cells

The Examiner asserts that the specification does not reasonably provide enablement for the diagnosis of a tumor or estimating the risk to develop a metastasis or recurrence.

In Giesing et al., 2009, it is stated that the AOX test was tumor predicting with a sensitivity of 86 %, specificity of 82 %, positive predictive value of 69 %, negative predictive value of 92 %, accuracy of 83 % and odds ratio of 28 (page 1 under "Results"). Each of the three genes was identified as a highly significant predictor of prostate cancer (page 8, 3rd column, last paragraph). Sustained overexpression of SOD2 (i.e. MNSOD) and GPX1 accounted as risk factors for distant tumor recurrence (page 1, sentence bridging 2nd and 3rd column). The AOX expression level allowed the identification of patients with high progression risk (page 1, 3rd column).

The fact that said results and statements were accepted for publication in a pre-reviewed journal illustrates the fact that a skilled person would have known how to use the recited methods for the diagnosis of a tumor or estimating the risk to develop a metastasis or recurrence.

9. The state of the art and any unpredictability

The Examiner asserts that the state of the art shows some unpredictability. Pusztai and Hess are cited by the Examiner to argue that the most appropriate validation for using gene expression analysis as a classification tool is testing the predictor on independent sets of cases.

The method of the present invention has, in fact, been tested on independent sets of cases, i.e., group A (patients with a clinically diagnosed tumor), group B (patients without a clinically diagnosed tumor), group C (patients after radical prostatectomy with low progression risk) and group D (patients after radical prostatectomy with high risk for progression). See Giesing et al., 2009 and Prof. Giesing's declaration of record.

Shalon et al. are cited by the Examiner to argue that the larger the number of individuals tested, the more significant the remaining differences in gene expression become.

There can be no doubt that the data presented in Giesing et al., 2009 as well as the declaration is statistically meaningful data.

Kroese et al. are cited by the Examiner to argue that genetic tests are heterogenous in nature and the exact characteristics of a particular genetic test to be evaluated must be tightly

defined. Kroese defines a genetic test as the analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes. Kroese's focus is on the concept of a gene test, defining that as one based on the analysis of human DNA using a variety of different technologies (Kroese et al., 2004, page 475, right-hand column, first full paragraph).

The present invention, however, does not relate to detecting heritable disease-related genotypes, mutations, phenotypes, or karyotypes for clinical purposes. The method of the present invention is about determining gene expression. Whereas Kroese states that the inability to identify all disease-related mutations make it difficult to estimate clinical validity of many genetic tests (Kroese et al., page 479, right-hand column, first paragraph under "Conclusion") such limitations do not account for the method of the present invention. Kroese et al. therefore is simply irrelevant.

Golub et al. are cited by the Examiner to assert the unpredictable nature of extrapolating gene expression differences to a method of class prediction.

Applicants respectfully disagree with that assertion. Golub et al. actually demonstrate the feasibility of cancer classification based solely on gene expression monitoring and suggest a general strategy for discovering and predicting cancer classes (Golub et al., page 531, abstract). In particular, Golub et al. state that the methodology of class prediction can be applied to any measurable distinction among tumors (page 533, paragraph bridging left and middle columns). Of course, not any gene expression differences will be useful for class prediction and therefore Golub suggests the use of a two-step procedure to test the validity of gene expression levels as predictors.

Applicants submit that the clinical utility of the method of the present invention for diagnosing tumors and estimating the risk to develop a metastasis or a recurrence has been validated (see, for instance, Giesing et al., 2009).

Seven et al. is cited by the Examiner to argue that the expression of antioxidant enzymes may vary according to the type of cancer or tissue, resulting in controversy in the literature.

However, neither Seven nor the literature reviewed by Seven is concerned with disseminated cancer cells. Applicants note that disseminated cancer cells are independent

from the tumor entity they are derived from and it is stated in Giesing et al., 2009 that their findings suggest the general applicability of the AOX-test system (page 2, middle column).

Applicants respectfully submit that the working examples described in the specification, the results reported in Prof. Giesing's declaration and the data presented in Giesing et al., 2009 corroborate that the method of the present invention is enabled over the entire scope claimed.

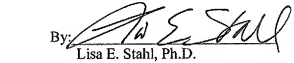
In view of the above, Applicants respectfully request reconsideration and withdrawal of the enablement rejection.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions related to this response, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney at the below-listed telephone number concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,
BACON & THOMAS, PLLC

By: 
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